

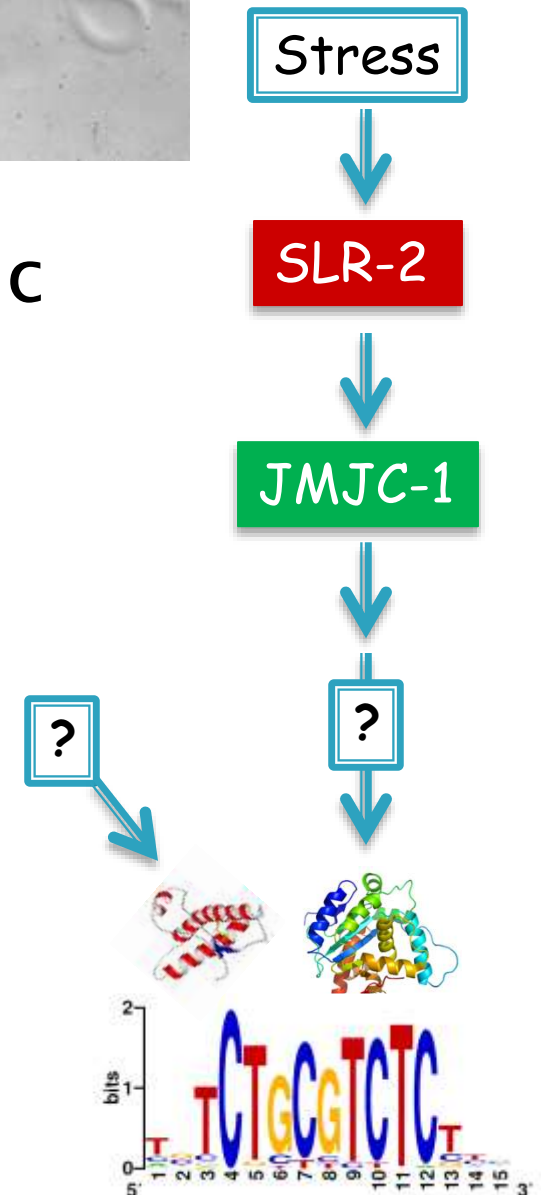
Elucidating the ESRE Stress Response Network

Anna Justis
Dr. David Fay, Mentor
Honors Program Senior Project

The ESRE Pathway in *C. elegans*

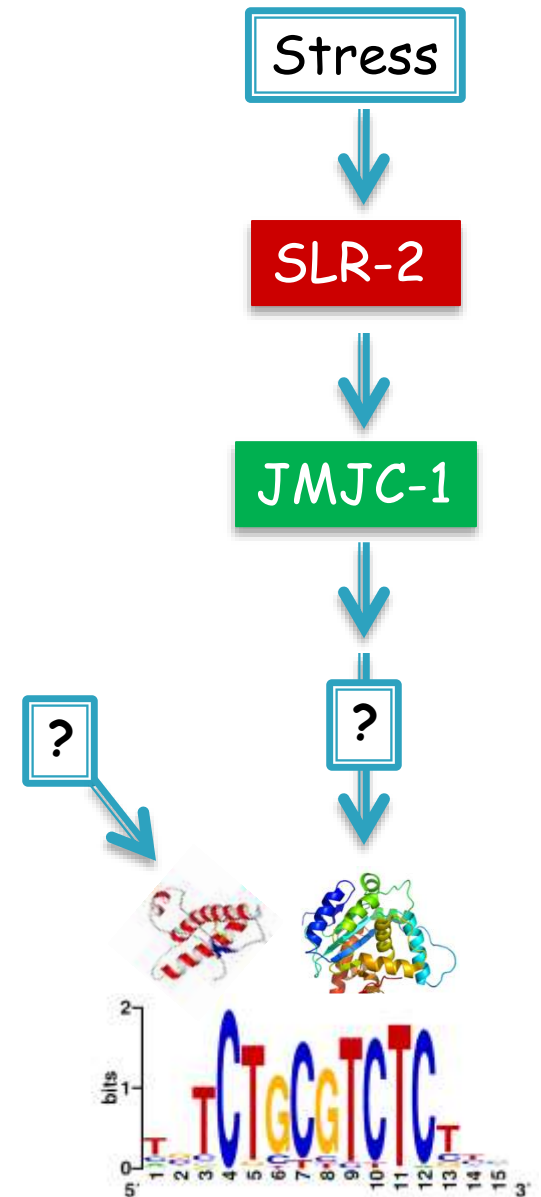


- ▶ Activated by heat shock, osmotic stress, redox stress, ethanol, & bacterial pathogen exposure
- ▶ Slr-2 and jmjc-1 are upstream participants
- ▶ Acts through a promoter motif termed ESRE (for ethanol and stress response element)

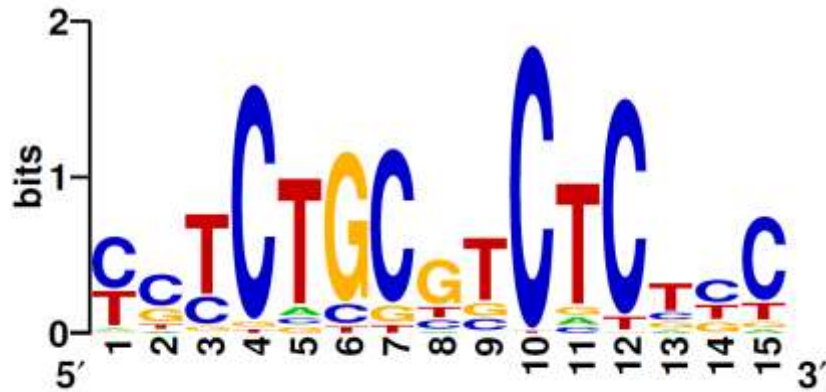
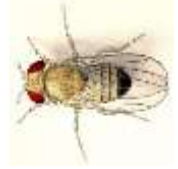
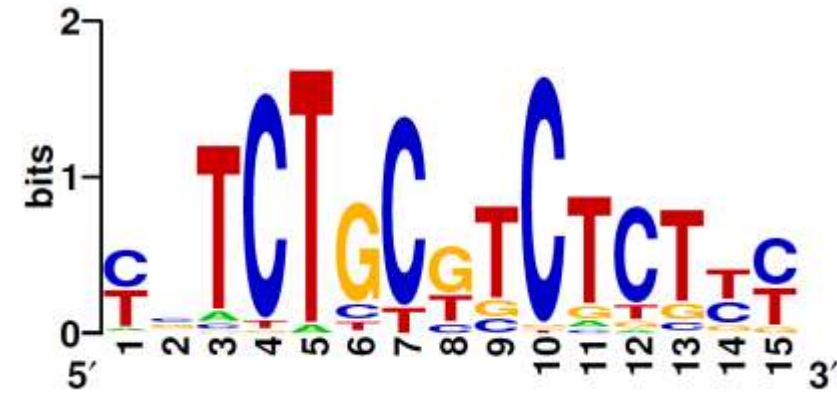


Research Questions

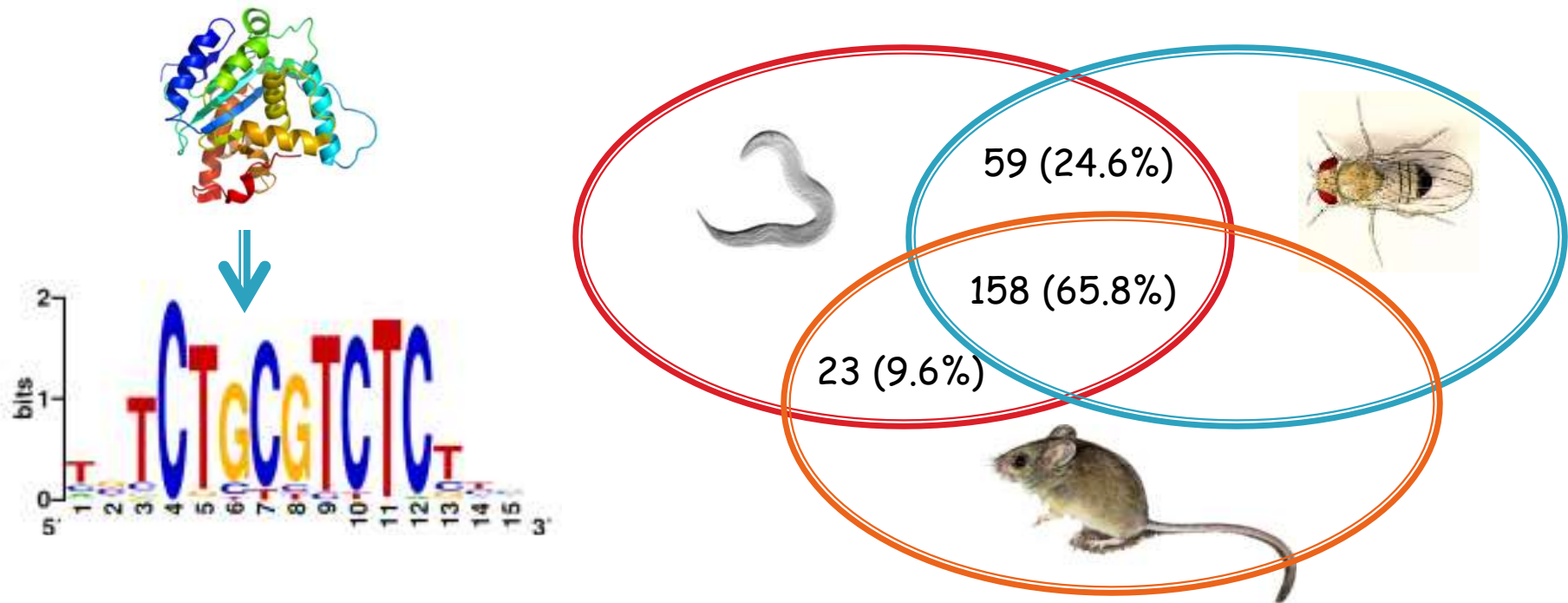
- ▶ How does the ESRE pathway work?
- ▶ Who are the players?
 - Identify the EBP (ESRE-binding protein)
 - Identify other participants in the ESRE pathway



- ▶ The ESRE motif sequence, *slr-2*, and *jmjc-1* are highly conserved among worms, flies, and mammals

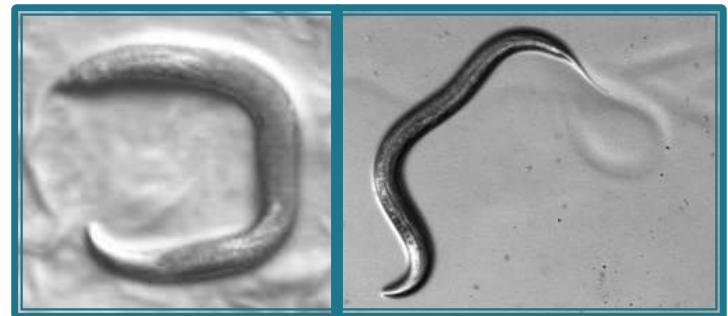


ESRE pathway participants are likely also conserved



RNAi Screen

- ▶ Double-stranded RNA used to knock down one gene at a time
- ▶ 3xESRE::GFP, *rol-6* reporter to visualize ESRE driven gene expression

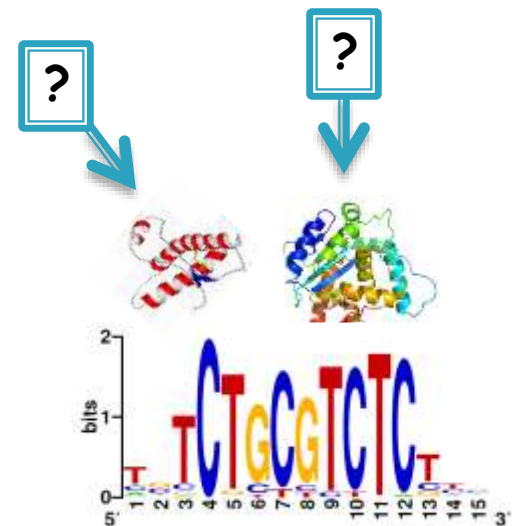


Roller

Non-Roller

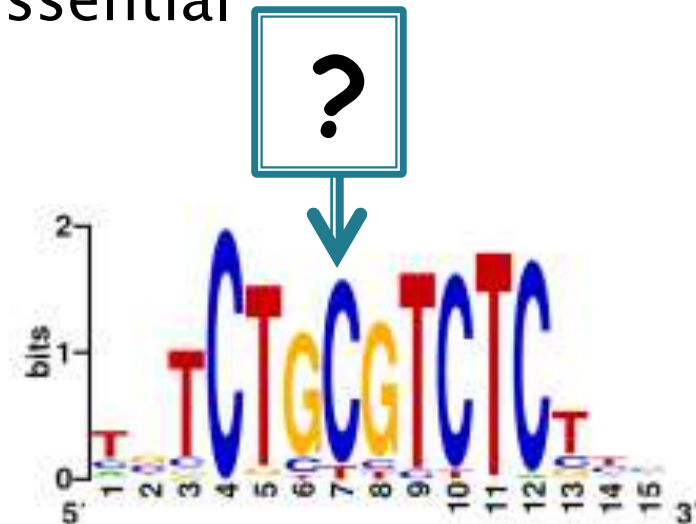
RNAi Screen

- ▶ Tested 217 clones of evolutionarily conserved **transcription factors**
- ▶ Tested 173 clones of known **signaling molecules**



RNAi Screen Results

- ▶ C08B1 1.3 RNAi knocks down 3xESRE::GFP expression by 2-fold
 - SWI/SNF nucleosome remodeling complex component
- ▶ No other genes identified from screen
 - Incomplete knockdown
 - ESRE pathway components are essential
 - Functional redundancy
 - A complex binds ESRE

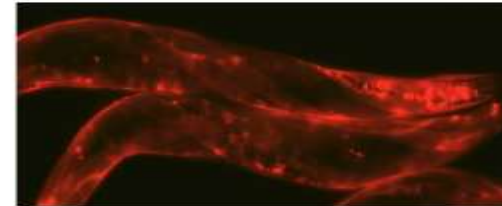


Forward Genetic Screen

- ▶ Parent strain:

3xESRE::GFP rol-6; dop-3::RFP; lin-2
3xESRE::GFP rol-6 dop-3::RFP lin-2

Wild Type



20°C

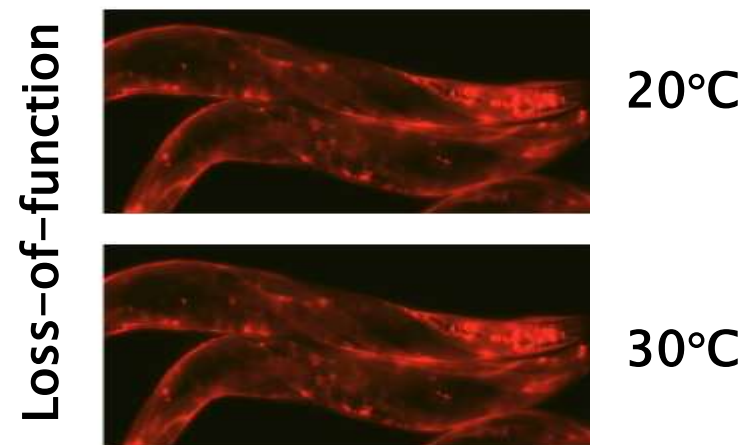
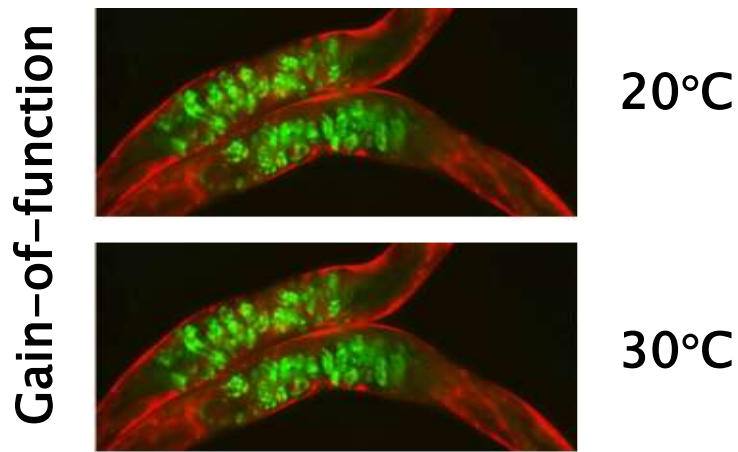


30°C

- ▶ Random mutagenesis by ENU (*N*-ethyl-*N*-nitrosourea)
- ▶ Look for interesting mutant phenotypes

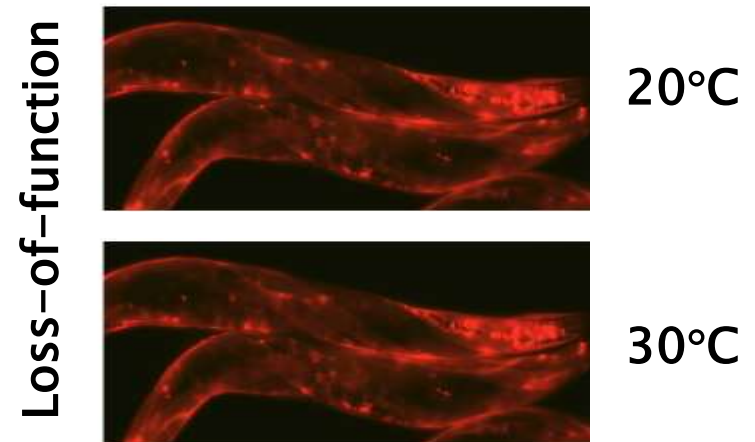
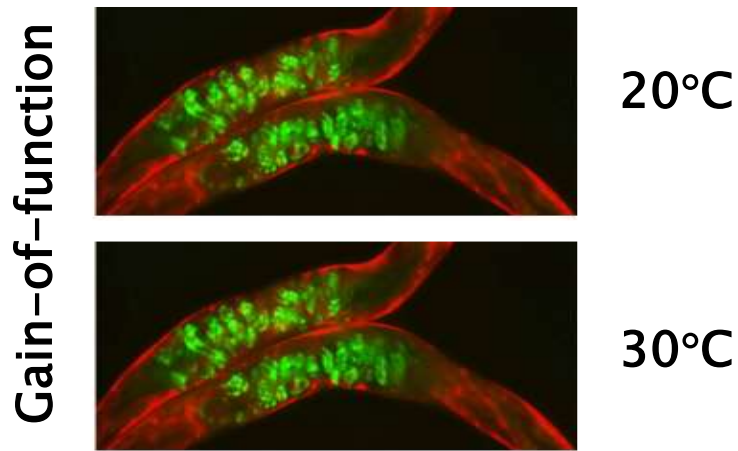
Forward Genetic Screen

- ▶ Gain-of-function mutations
 - Constitutively expressed 3xESRE::GFP
“green w/o heat shock”
- ▶ Loss-of-function mutations
 - “reduced green after heat shock”



Over 200 candidate ESRE pathway mutants identified

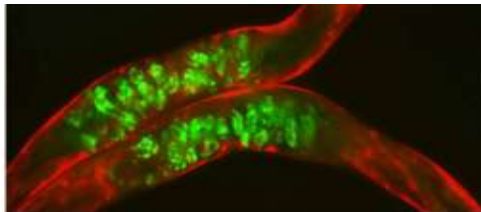
- ▶ Is the phenotype caused by a single mutation?
- ▶ Is the mutation linked to the reporter array?
- ▶ Does the mutation also effect other ESRE-driven reporters?



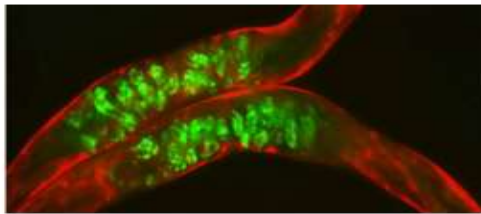
Forward Genetic Screen Results

- ▶ On-going analysis....
- ▶ So far:
 - 2 GOF mutants
 - 2 LOF mutants

Gain-of-function

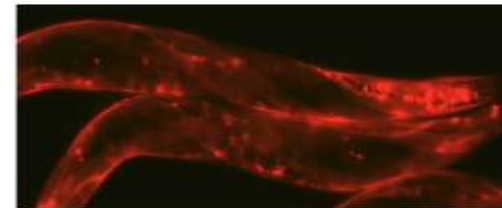


20°C



30°C

Wild Type

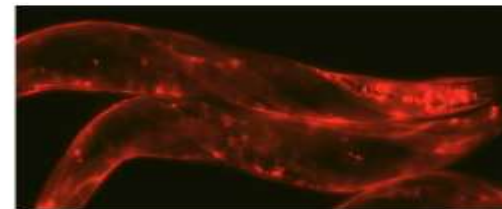


20°C

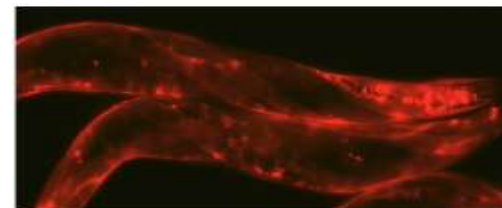


30°C

Loss-of-function



20°C



30°C

Acknowledgements

Dr. David Fay

Aleksandra Kuzmanov

John Yochem

Questions?